



Milk energy output during peak lactation in shaved Swiss mice

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ABSTRACT

The limits to sustainable energy intake (SusEI) are very important because they define an envelope within which many aspects of animal performance are constrained. It has previously been suggested that Swiss mice may be constrained peripherally by the mammary gland, in contrast to the heat dissipation limits hypothesis. To distinguish between the two ideas, we dorsally shaved Swiss mice at early lactation, and examined the energy intake, resting metabolic rate (RMR), litter size and mass, milk energy output (MEO), serum prolactin levels (PRL) and suckling behavior of shaved mothers and non-shaved controls. Dorsal fur removal significantly increased energy intake and RMR, but did not have significant effects on litter mass, MEO, PRL and suckling behavior. These data were inconsistent with the heat dissipation limitation hypothesis and provided support for the peripheral limitation hypothesis, i.e. SusEI was more likely peripherally caused by the capacity of the mammary gland to produce milk. The inconsistent responses to sustainable limits suggested that the limitations on SusEI during peak lactation might be not the same in all species or even between different strains of mice.

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1. Introduction

The limits to sustainable energy intake (SusEI) are of importance because they define an upper bound that constrains many aspects of animal performance, including reproductive output, foraging behavior and thermoregulatory capabilities [1–9]. Lactation is the most energetically demanding period that small mammals experience, and thus is widely used to examine the limits to SusEI [9–16].

It is believed that the limits to SusEI are likely to be intrinsically set by aspects of an animal's physiology [9]. The factors limiting SusEI are probably not imposed centrally by the energy-supply system, for instance, the capacity of the gastrointestinal tract and associated organs to acquire, process, and absorb energy, generally known as the “central limitation hypothesis”. Instead, limits may be caused peripherally by the energy consuming machinery, such as the capacity of mammary glands to produce milk which is called the “peripheral limitation hypothesis” [1,2,4,9–11,14,15,17–24]. However, it seems difficult to explain some data using this peripheral limitation hypothesis. For example, MF1 mice at 30 °C had a lower asymptotic food intake during peak lactation and raised fewer and smaller pups than those lactating at 21 °C and 8 °C [8,14,25]. These females lactating at 30 °C also reduced milk energy out (MEO) [8,14,25]. Additionally, dorsally shaved MF1 females increased their food intake during peak lactation, paralleled by an increase in MEO [26]. These data argued against the peripheral limitation hypothesis and provided

support for a new hypothesis, i.e. the heat limit hypothesis proposed by Krol and Speakman in 2003 [8,9,25,26]. Here the SusEI during peak lactation was suggested to be constrained by the capacity of animals to dissipate body heat. Under this hypothesis, fur-removal manipulation enhanced thermal conductance of animals, broke the limitations on SusEI, and thus allowed the animals to increase both food intake and reproductive output [8,9,25,26].

However, Hammond et al. (1996) manipulated Swiss mice by surgically removing some mammary tissue. They found that mice were unable to upregulate milk production in the remaining tissue which would be predicted from the heat dissipation limit hypothesis [11]. Zhao and Cao (2009) previously performed a shaving experiment in Swiss mice and found no support for the heat dissipation limit hypothesis [24]. Zhang and Wang (2007) exposed female Brandt's voles with their litters to a cold temperature (5 °C) throughout lactation. The mothers lactating in the cold had higher energy intake and greater energy exported for resting metabolic rate (RMR) and nonshivering thermogenesis (NST), but raised smaller litters than the mothers lactating at room temperature [22]. These data were consequently more consistent with the peripheral limitation hypothesis whereby SusEI was defined by the summed metabolic demands of contributing metabolic processes. However, Wu et al. (2009) exposed Brandt's voles to high temperatures and found that the responses were consistent with the heat dissipation limits hypothesis, particularly for those raising larger litters [27]. The reasons for this difference between the different species, even between the strains of a species, remain uncertain.

In the present study we aimed to repeat the shaving experiment performed by Krol et al. (2007), but instead used Swiss mice to establish

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if this strain was limited by the capacity of mammary gland to produce milk or the heat dissipation capacity. This would enable us to distinguish whether there are strain-specific responses to shaving. During lactation we examined the effects of shaving on the energy intake, litter size and mass, RMR, MEO and serum prolactin (PRL) levels. Finally, the time spent on suckling pups during peak lactation was recorded in both shaved and non-shaved females.

2. Materials and methods

2.1. Animals and experimental protocol

All experimental procedures were in compliance with the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Virgin female Swiss mice aged 8–10 weeks were housed individually in plastic cages (29×18×16 cm) with fresh sawdust bedding. They were maintained at $23 \pm 1^\circ\text{C}$ on a 12:12 h light:dark cycle (lights on at 0700 h) and were given free access to standard rodent chow (produced by the Animal Center of Shandong Province) and water. Forty females were paired with males for 11 days, after which the males were removed. Thirty eight mice became pregnant and gave birth. On day 7 of lactation, the lactating females were separated into either control ($n = 19$) or shaved groups ($n = 19$) that were matched for litter size on day 6. On day 7 of lactation, all females from both control and shaved groups were anaesthetized, and 19 females in the shaved group were shaved dorsally as described by Krol et al. (2007) [26] (RFJZ-838 Hair Clipper, ShenBa Co. China) to remove approximately 0.385 ± 0.091 g of fur. All mice were housed at $23 \pm 1^\circ\text{C}$ throughout lactation. All pups were weaned on day 17 of lactation. The female body mass and food intake, as well as litter size and litter mass were measured on a daily basis as described previously (Zhao and Cao, 2009). The asymptotic food intake during the peak lactation was calculated as the mean daily food intake between days 11–16 of lactation as no significant changes were observed over this period by repeated measurements (RM-ANCOVA, control, $F_{5, 180} = 0.41$, $p > 0.05$, body mass, $F_{1, 36} = 7.01$, $p < 0.05$).

2.2. Energy intake and digestibility

Energy intake and digestibility during peak lactation were measured over the 2 days of lactation (days 13–14, control, $n = 15$, shaved, $n = 12$). The peak lactation usually refers to a period of maximal energy demands of mothers for supporting their offspring, which generally occurs on the late lactation for rodents. So the measurements of maximal sustained energy intake or expenditure during peak lactation in laboratory mice are usually carried out between days 11–16 of lactation [2,3,10,11,14,15,18,24,26]. As described previously [28–31], the spillage of food mixed with bedding (<2%) and feces were collected from each animal over this period and separated manually and then dried at 60°C to constant mass. The gross energy contents of the food and feces were determined by a Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA). Dry matter intake (DMI), gross energy intake (GEI), digestible energy intake (DEI), metabolizable energy intake (MEI) and apparent energy assimilation efficiency (digestibility) were calculated as follows [28–31]:

$$\begin{aligned}\text{DMI (g/d)} &= \text{food intake (g/d)} \times \text{dry matter content of food (\%)} - \text{dry spillage of food;} \\ \text{GEI (kJ/d)} &= \text{DMI (g/d)} \times \text{gross energy content of food (kJ/g);} \\ \text{DEI (kJ/d)} &= \text{GEI} - [\text{dry feces mass (g/d)} \times \text{gross energy content of feces (kJ/g)}]; \\ \text{MEI (kJ/d)} &= \text{DEI (kJ/d)} - \text{urine energy (kJ/d);} \\ \text{Digestibility (\%)} &= \text{DEI/GEI} \times 100\%.\end{aligned}$$

Urinary energy loss was not measured and was assumed to be 2% of the DEI [28,29,32].

2.3. Resting metabolic rate (RMR)

RMR was quantified as the rate of oxygen consumption in a closed circuit respirometer as described previously [33–35]. Briefly, the chamber was 3.6 L in size and CO_2 and water in the chamber were absorbed with KOH and silica gel, respectively. The metabolic chamber temperature was controlled within $\pm 0.5^\circ\text{C}$ by immersion in a water bath. RMR was determined at a thermoneutral temperature of $30 \pm 0.5^\circ\text{C}$ [36]. The mice were deprived of food for 5 h, but given free access to water before entering the metabolic chamber. After 1 h adaptation to the chamber, oxygen consumption was recorded for 60 min at 5 min intervals. RMR was defined as the average of the lowest two consecutive recordings and was corrected to standard temperature and air pressure (STP) conditions. Oxygen consumption (mlO_2/h) was converted to energy expenditure (kJ/d), using the equation of Weir [5,15,37]. All measurements were made between 14:00 and 18:00 on day 17 of lactation.

2.4. Behavioral observation

Suckling behavior observations were made on day 14–15 in 10 control females with their litters (range 10–13) and 10 shaved females with the same litter size range as controls. Suckling behavior was observed as described previously by Speakman et al. [38]. Briefly, each female was observed in sequence, and a series of 20 cages were observed at a batch of 1 min, in which each cage was observed for 3 s. Within the observation period, the dominant behavior was recorded as either suckling or non-suckling for each mother. If the mothers did not suckle pups whatever they did or the pups were stopped from suckling, the behavior of females was then defined as non-suckling behavior. All the observations were made during the light phase (0700 h–1900 h). Thus, in total, each cage was observed 720 times over a period of 12 h. The suckling duration of each mother was calculated as the cumulative suckling behavior in each suckling bout.

2.5. Milk energy output (MEO)

MEO during peak lactation (day 13–14, control, $n = 15$, shaved, $n = 12$) was evaluated, as described previously, from energy budget of the litter (E_L) [25]. The pups depend entirely on milk, and energy total (E_L) was the sum of energy allocated to respiration (R) and energy accumulated as new tissue. R was predicted from the pup body mass using the relationship between RMR and body mass. It assumed that $R = 1.4 \times \text{RMR}$ to take the energetic costs of activity of pups into account. The equation used was [25]:

$$\text{MEO} = [(7.28 + 0.71 \times LM) \times CF_{\text{act}} + LMI \times GE_{\text{pups}}] \times 100/d_{\text{milk}}.$$

Where, MEO (kJ/d) was milk energy output; LM (g) was the litter mass on day 14 of lactation; CF_{act} was the correction factor ($CF_{\text{act}} = 1.4$), the mean ratio of daily energy expenditure to RMR; GE_{pups} (kJ/g wet mass) was the gross energy content of pups. The mean values of GE_{pups} used in this formula for control and shaved groups were determined using a Parr 1281 oxygen bomb calorimeter from 8 pups from the control group and 8 from the shaved group (control, 6.39 ± 0.06 kJ/g wet mass, shaved, 6.48 ± 0.22 kJ/g wet mass, $F_{1, 13} = 0.1$, $p > 0.05$). LMI (g/d) was the litter mass increase between days 13 and 14 of lactation. d_{milk} was the apparent digestibility of milk ($d_{\text{milk}} = 96\%$) [25,39].

2.6. Serum prolactin (PRL)

After RMR measurement on day 17 of lactation, the females were returned to the cages, and were still given free access to the pups. One hour later, the females were removed from the cages when they were suckling pups, and sacrificed immediately by decapitation between 1700 h and 1830 h. Trunk blood was collected for PRL measurement. Serum was separated from each blood sample by centrifugation and

stored at -75°C . Serum PRL levels were quantified by radioimmunoassay using RIA kits (China Institute of Atomic Energy, Beijing). This RIA kit was validated and used for Swiss mice following the standard kit instructions. Intra- and inter-assay coefficients of variation were 4.3% and 7.6% for PRL.

2.7. Statistics

Data were expressed as mean \pm SE and analyzed using SPSS 13.0 statistic software. Repeated-measures analysis of variance or covariance (RM-ANOVA or RM-ANCOVA) was used to determine the significance of changes in body mass, food intake, litter size and mass over time with female body mass added as covariate where required. Direct comparisons of the body mass of control and shaved females were made using two-sample *t*-tests [15,20,21]. The effects of shaving on DMI, GEI, DEI, MEI, digestibility, litter size and mass, RMR, MEO, PRL as well as suckling behavior were evaluated using ANCOVA with female body mass, litter mass, or both as covariates where appropriate. To correct for body mass, we calculated residuals for DEI, MEO from the least-squares regression lines on female body mass. Relationships between the residuals were described using Pearson product-moment correlation coefficients [9,15,20,21,40]. For percentage data, arcsine-square-root transformation was performed prior to analysis to normalize the data. The level of significance was set at $p < 0.05$.

3. Results

3.1. Female body mass and food intake

There was no significant group difference in body mass during early lactation (day 3, $t_{36} = 0.33$, $p > 0.05$) or before the fur-shaving

manipulation (day 6, $t_{36} = 0.75$, $p > 0.05$). Shaved females did not differ significantly in body mass from control mice on day 7 and thereafter (day 7, $t_{36} = 0.96$, $p > 0.05$; day 17, $t_{36} = 0.75$, $p > 0.05$, Fig. 1A). Both control and shaved females increased the food intake over the period of lactation (days 3–17, RM-ANCOVA, control: group, $F_{14, 238} = 1.94$, $p < 0.05$, body mass, $F_{1, 17} = 17.18$, $p < 0.01$; shaved: group, $F_{14, 238} = 2.00$, $p < 0.05$, body mass, $F_{1, 17} = 9.11$, $p < 0.01$). Between the two groups, the shaved females had significantly higher food intake on day 11, 13, 14 and 17 (day 11, group, $F_{1, 35} = 5.54$, $p < 0.05$, body mass, $F_{1, 35} = 5.89$, $p < 0.05$, Fig. 1B). The asymptotic food intake of shaved females averaged 22.88 ± 0.74 g/d, which was significantly higher than the 21.03 ± 0.70 g/d of the control group (group, $F_{1, 35} = 9.38$, $p < 0.01$, body mass, $F_{1, 35} = 9.63$, $p < 0.01$).

3.2. Litter size and litter mass

Before the shaving manipulation, litter size averaged 10.16 ± 0.48 (range from 7–14) for controls and 10.63 ± 0.51 (range from 7–16) for shaved females (day 6, $p > 0.05$, Fig. 1C). The litter mass of controls was 44.53 ± 1.97 g and that of shaved females was 45.94 ± 2.16 g (day 6, $p > 0.05$, Fig. 1D). At weaning, neither control nor shaved females showed significant difference in litter size (9.89 ± 0.48 vs 10.11 ± 0.51 , $p > 0.05$) or litter mass (81.13 ± 2.44 g vs 85.06 ± 2.48 g, $p > 0.05$).

3.3. Energy intake and digestibility

Shaved females had significant higher DMI during peak lactation than control females (group, $F_{1, 23} = 12.1$, $p < 0.01$; body mass, $F_{1, 23} = 0.93$, $p > 0.05$; litter mass, $F_{1, 23} = 5.31$, $p < 0.05$). The GEI of control females was 317.5 ± 9.4 kJ/d and that of shaved females was 355.5 ± 9.4 kJ/d. There was a 12% increase in GEI in the shaved group in comparison

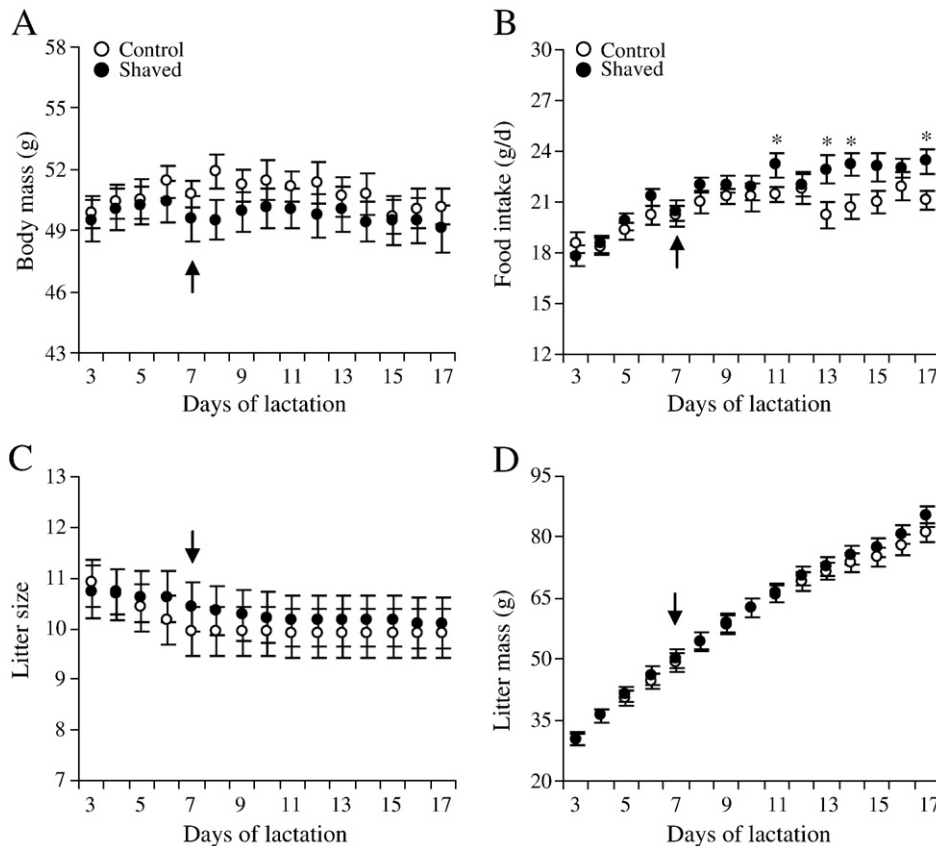


Fig. 1. Changes of the female body mass (panel A), food intake (panel B), litter size (panel C) and litter mass (panel D) throughout lactation in control (open circles) and shaved Swiss mice (filled circles). The arrows indicate that the females in the shaved group were dorsally shaved on day 7 of lactation. The values are mean \pm SE. *, significant difference between control and shaved females ($p < 0.05$).

with control females (group, $F_{1, 23} = 12.1$, $p < 0.01$; body mass, $F_{1, 23} = 0.93$, $p > 0.05$; litter mass, $F_{1, 23} = 5.31$, $p < 0.05$, Fig. 2A). DEI of shaved females was 14% higher than that of control females (group, $F_{1, 23} = 11.0$, $p < 0.01$; body mass, $F_{1, 23} = 0.03$, $p > 0.05$; litter mass, $F_{1, 23} = 6.73$, $p < 0.05$, Fig. 2B). Consistently, shaved females had significantly higher MEI than controls (control, 222.7 ± 6.0 kJ/d; shaved, 253.4 ± 8.4 kJ/d, for the statistics, please see the DEI). There was no significant difference in digestibility between the two groups ($F_{1, 23} = 0.3$, $p > 0.05$, Fig. 2C). DEI was significantly positively correlated with litter mass for control females ($r = 0.71$, $p < 0.01$), whereas the correlation was not significant for shaved mothers ($r = 0.44$, $p = 0.15$, Fig. 3A).

3.4. Milk energy output (MEO)

The MEO during peak lactation was 102.7 ± 4.4 kJ/d for controls and 107.5 ± 3.7 kJ/d for shaved females, which was not significantly different (group, $F_{1, 23} = 0.40$, $p > 0.05$; body mass, $F_{1, 23} = 4.90$, $p < 0.05$; litter mass, $F_{1, 23} = 25.41$, $p < 0.01$, Fig. 4A). The power to detect a significant difference in MEO at this effect size and sample size was 94.5%. Both control and shaved females showed positive correlation between MEO and litter mass (control, $r = 0.88$, $p < 0.01$; shaved, $r = 0.84$, $p < 0.01$, Fig. 3B). The correlation between residual DEI and MEO was significant in shaved mice ($r = 0.80$, $p < 0.01$), which was not observed in controls ($r = 0.38$, $p = 0.16$, Fig. 5).

3.5. Resting metabolic rate (RMR)

The RMR of control females was 63.7 ± 1.9 kJ/d and in shaved females was 74.1 ± 2.8 kJ/d. RMR was 16% higher in the shaved group (group, $F_{1, 23} = 20.4$, $p < 0.01$, body mass, $F_{1, 23} = 1.42$, $p > 0.05$; litter mass, $F_{1, 23} = 9.61$, $p < 0.01$, Fig. 4B).

3.6. Suckling behavior

Shaved females showed the same number of suckling bouts at peak lactation as control females (group, $F_{1, 17} = 0.03$, $p > 0.05$; litter mass, $F_{1, 17} = 0.16$, $p > 0.05$, Table 1). Cumulative suckling duration over the 12 h light phase and the mean suckling duration of shaved females were also similar to that of control females.

3.7. Serum prolactin (PRL)

The serum PRL levels of shaved females did not significantly differ from that of control females (group, $F_{1, 34} = 0.44$, $p > 0.05$, litter mass, $F_{1, 34} = 15.58$, $p < 0.01$; body mass, $F_{1, 34} = 0.97$, $p > 0.05$, Fig. 4C). The serum PRL levels were positively correlated with DEI and MEO in the control group (DEI and PRL, $r = 0.61$, $p < 0.05$, Fig. 6A; MEO and PRL, $r = 0.57$, $p < 0.05$, Fig. 6B), whereas the correlations were not

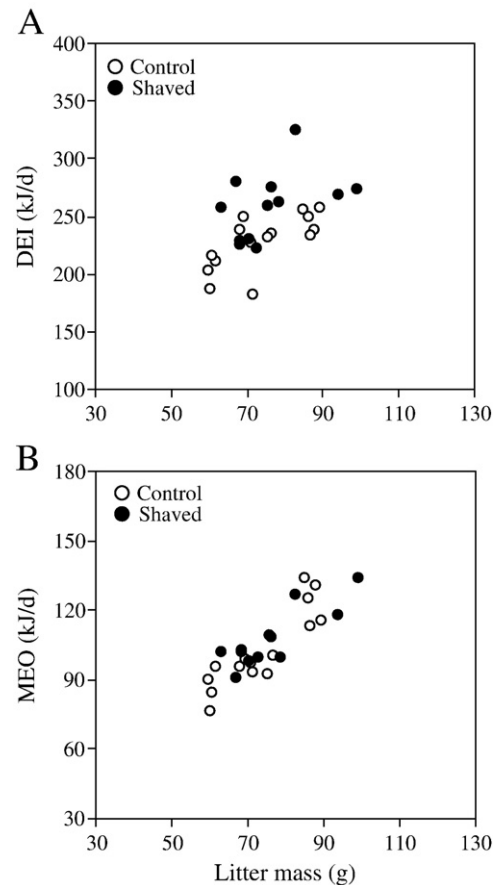


Fig. 3. Digestible energy intake (DEI, panel A) and milk energy output (MEO, panel B) as a function of litter mass for control (open circles) and dorsally shaved Swiss mice (filled circles). Correlation between DEI and litter mass was significant for control females ($r = 0.71$, $p < 0.01$) but not significant in the shaved group ($r = 0.44$, $p = 0.15$). MEO was correlated with litter mass in both groups (control, $r = 0.88$, $p < 0.01$; shaved, $r = 0.84$, $p < 0.01$).

significant in the shaved group (DEI and PRL, $r = 0.26$, $p > 0.05$; MEO and PRL, $r = 0.40$, $p > 0.05$). The PRL levels were positively correlated with litter size in both groups (control, $r = 0.76$, $p < 0.01$; shaved, $r = 0.67$, $p < 0.01$, Fig. 7A). A significant correlation between PRL levels and litter mass was found in controls ($r = 0.74$, $p < 0.01$), but was not observed in shaved females ($r = 0.44$, $p = 0.06$, Fig. 7B). Both control and shaved females showed positive correlation between PRL and mean suckling time (control, $r = 0.82$, $p < 0.01$; shaved, $r = 0.89$, $p < 0.01$, Fig. 7C).

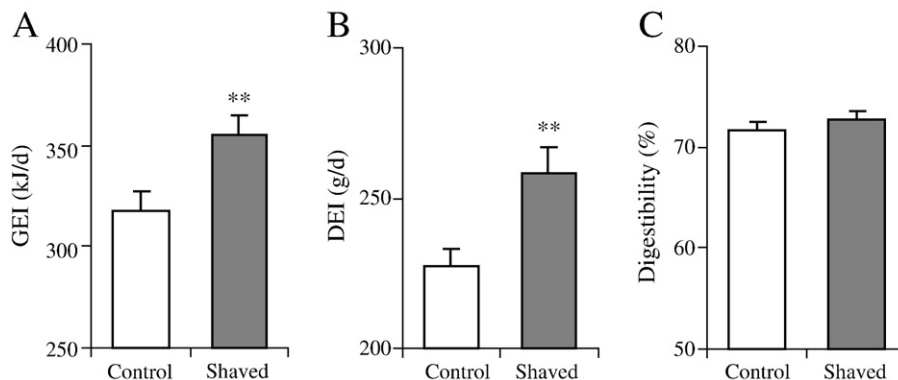


Fig. 2. Gross energy intake (GEI, A), digestible energy intake (DEI, B) and digestibility (C) during peak lactation in control (open columns) and dorsally shaved Swiss mice (filled columns). The values are mean \pm SE. **, significant difference between control and shaved females ($p < 0.01$).

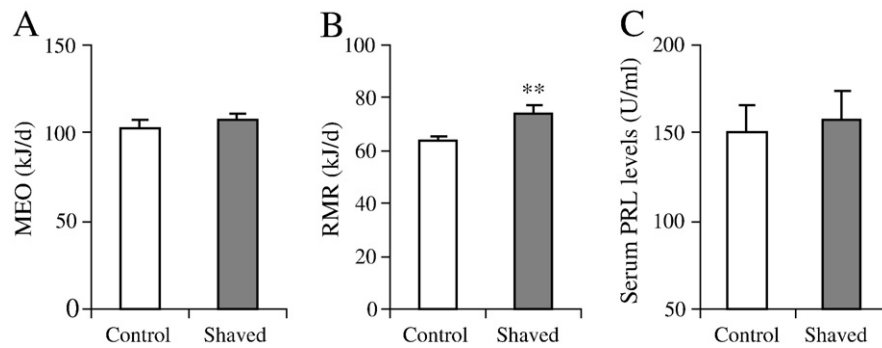


Fig. 4. Energy milk output (MEO, panel A), resting metabolic rate (RMR, panel B) and serum prolactin levels (PRL, panel C) in control (open columns) and dorsally shaved Swiss mice (filled columns). The values are mean \pm SE. **, significant difference between control and shaved females ($p < 0.01$).

4. Discussion

In the present study, Swiss mice had no significant change in body mass and did not respond to dorsal fur-removal manipulation by raising larger litters of a larger litter mass. However, shaved females did increase their food intake during peak lactation beyond that observed in non-shaved controls. Consistently, we also found a significant increase in energy intake during peak lactation in shaved females. Shaved mice increased their food intake by 12% and assimilated 14% more energy than unshaved females. Krol et al. (2007) reported similar results in MF1 mice, in which shaved mothers had significantly higher food intake than unshaved females [26]. The increase in food intake reported by Krol et al. was almost identical, showing fur manipulation had a similar effect on heat loss in both studies. As there was no significant difference in digestibility between shaved and non-shaved females, it seemed the females were not capable of increasing digestibility to compensate for the high energy demands during lactation, but were more likely able to increase energy intake. We also showed no significant correlations between DEI, MEO and litter mass in shaved mice, suggesting that the increase in energy intake was likely to be contributing to the increased RMR caused by dorsal fur-removal treatment, rather than allocated to the energy exported for milk. This supports a previous suggestion that lactating animals were not limited centrally by the alimentary tract but might be peripherally by the mammary glands [2,3,9–16,20,21,24,38]. If this idea was correct, an elevated energy intake would not be translated to milk energy output and consequently should not result in the increase in reproductive performance of an animal.

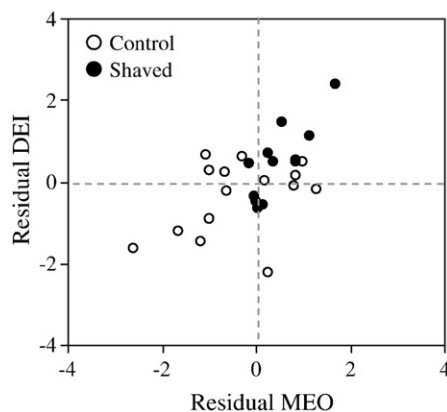


Fig. 5. Residual digestible energy intake (DEI) as a function of residual milk energy output (MEO) for control (open circles) and dorsally shaved Swiss mice (filled circles). The correlation was significant for shaved mice only ($r = 0.80$, $p < 0.01$).

In the present study, the pups supported by shaved mothers showed a similar resting or suckling time to those pups raised by non-shaved mothers (personal observation). So we assumed that the “correction factor” for activity of the pups would not be affected by the shaving of the mothers, and thus was assumed to be suitable for the energy budget approach to quantify milk production. The MEO calculation was based on the mice under thermoneutral conditions, but here the mice were lactating at 23 °C (outside their thermoneutral zone) and were spending energy on thermoregulation. Therefore, MEO may have been underestimated. However, as all mice were exposed to the same temperature between shaved and non-shaved groups, the MEO were therefore presumed to be comparable between the two groups. The MEO of shaved females was not different to that of unshaved controls. There was also no significant difference in litter mass between shaved and unshaved females [24]. In agreement with data from Swiss mice, the volume of milk produced was greater in MF1 mice with heavier litters, whereas the energy content decreased with increasing volume and the energy provided in milk was not different over the range of 9–15 pups [15]. Some other rodents also failed to increase energy output in milk for large litters, such as house mice *Mus musculus* [41,42], rats *Rattus norvegicus* [43], and cotton rats *Sigmodon hispidus* [4]. It has previously been suggested that the mothers supporting large litters rapidly reduced milk production to the level of small litters, but the mothers raising small litters failed to increase their milk energy output when made to suckle a large litter [15,44]. These data provided support for the peripheral limitation hypothesis, i.e. SusEI was more likely peripherally caused by the capacity of the mammary glands to produce more milk.

The suckling stimulus is one of the primary factors stimulating oxytocin release, and also feeds back to prolactin release, thereby regulating milk production [9]. In the present study, serum prolactin levels were correlated with litter size, litter mass and suckling bouts. However, neither suckling bouts nor serum prolactin levels were significantly different between the shaved and non-shaved Swiss mice. During late lactation, the suckling pups might result in maternal hyperthermia in the nest, and therefore forced the female to discontinue suckling, which was suggested to be one of mechanisms

Table 1
Suckling behavior during peak lactation in control and shaved Swiss mice.

	Controls	Shaved mice	F	P
	n = 10	n = 10		
Mean litter size	11.2 \pm 0.3	11.1 \pm 0.3	0.10	0.75
Litter size range	10–13	10–13		
Suckling bouts (12 h)	10.9 \pm 0.7	11.0 \pm 2.0	0.03	0.89
Cumulative suckling duration (min/12 h)	452.0 \pm 30.0	454.0 \pm 30.1	0.05	0.88
Mean suckling duration (min)	42.0 \pm 2.1	50.3 \pm 6.4	1.14	0.30

Values are presented as mean \pm SE.

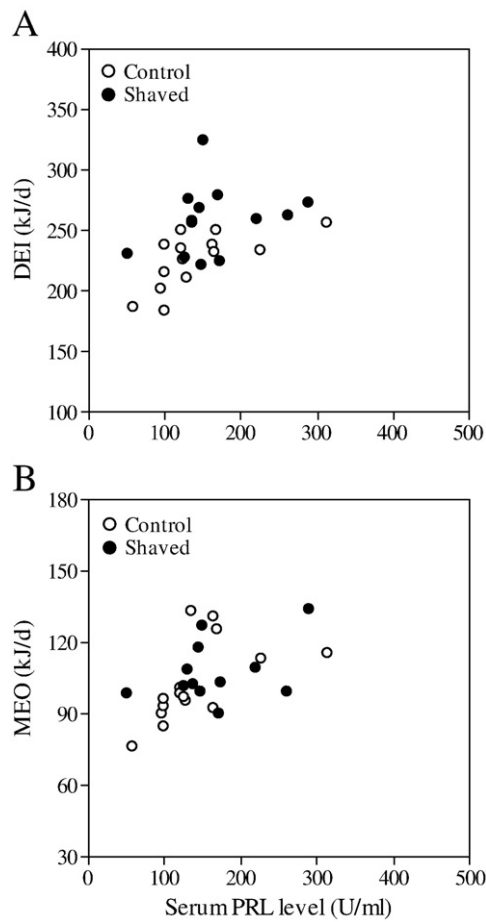


Fig. 6. Digestible energy intake (DEI, panel A) and milk energy output (MEO, panel B) as a function of serum prolactin levels (PRL) for control (open circles) and dorsally shaved Swiss mice (filled circles). The correlations were significant for controls (DEI and PRL, $r = 0.61$, $p < 0.05$; MEO and PRL, $r = 0.57$, $p < 0.05$), which was not observed in shaved females.

linking the heat dissipation capacity to lactation performance [9,45–47]. This possibility was supported by studies in rats, in which an increase in body temperature led to the disruption of maternal suckling behavior [48,49]. The relationships between body temperature, suckling bout and mammary glands performance were still unclear because MEO was not measured in these lactating rats. Additionally, morphine and naloxone combinations induced an increase in body temperature in rats, but this did not shorten their suckling bouts [50]. We also found correlations between serum prolactin levels and DEI and MEO, which was inconsistent with the prediction by heat dissipation limitation hypothesis. Thus, such an assumed mechanism linking heat dissipation capacity to lactation performance would appear as a paradox. It should be pointed out that a drawback of the present study was the lack of body temperature measurements, and therefore, more studies should be performed to carefully address this issue.

In contrast to Swiss mice, Krol and Speakman (2003) found that MF1 mice exposed to 8 °C had significantly higher food intake and reproductive performance during late lactation than those lactating at 30 °C [8,25]. Consistently, after exposed to high temperature (30 °C) Brandt's voles had a lower asymptotic food intake and raised lighter litter masses, particularly for those raising larger litters [27]. Moreover, dorsally shaved MF1 mice had significantly higher food intake during peak lactation and raised significantly heavier litter mass than unshaved mothers. These data suggested that cold exposure and fur removal increased the heat dissipation capacity of

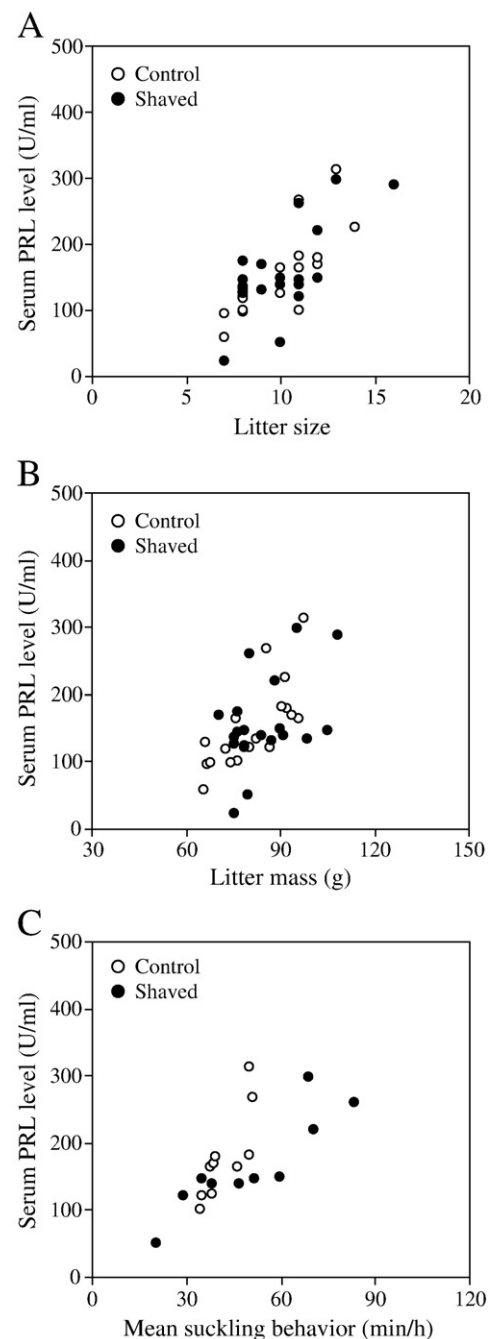


Fig. 7. Serum prolactin levels (PRL) as a function of litter size (panel A), litter mass (panel B) and mean suckling time (panel C) for control (open circles) and shaved mice (filled circles). The PRL levels were positively correlated with litter size and litter mass in both groups (litter size, control, $r = 0.76$, $p < 0.01$; shaved, $r = 0.67$, $p < 0.01$; litter mass, control, $r = 0.82$, $p < 0.01$; shaved, $r = 0.89$, $p < 0.01$). A significant correlation between PRL levels and litter mass was found in controls ($r = 0.74$, $p < 0.01$), but was not observed in shaved females ($r = 0.44$, $p = 0.06$).

the mothers, which led to significant elevation of food intake and milk production, and resulted in increases of reproductive performance [26]. Based on the heat dissipation limit idea, for MF1 mice the SusEI during peak lactation was likely constrained by the capacity of an animal to dissipate heat rather than limited by the capacity of mammary glands to produce milk, whereas this might not be the case for Swiss mice, suggesting different responses of animals to the limitations on SusEI between different rodent species or strains.

Why are the capacities of the mammary glands so different between the two strains? The exact reason remains unclear. Zhao and

Cao (2009) suggested that this difference might reflect that the relative positions of the limits on the capacities of the mammary glands to synthesize milk and the limits on heat dissipation capacity might not be the same in the different species or different strains of one species [24]. For example, for MF1 mice the food intake and reproductive output did not change in mothers made simultaneously pregnant while lactating or forced to support additional pups [15,20,21], which were consistent with both hypotheses. When the limitation on dissipating heat was relaxed under the condition of cold exposure or fur removal, the manipulated mice then extended their food intake further and raised heavier litters [8,9,25,26], suggesting that the heat dissipation capacity constraining SusEI might lie lower than the mammary glands. In Swiss mice, the females with five teats were unable to increase milk production to compensate for the lost production of the tissue that had been removed [11]. The females with manipulated larger litter size did not export more milk for the additional pups and finally weaned a lower pup body mass (Zhao et al. unpublished data). These data were consistent with that predicted by peripheral limitation idea and the heat dissipation hypothesis. In the later study, shaved females showed increases in thermal conductance and food intake during peak lactation, whereas did not increase milk energy output and raise heavier litters [24], suggesting that the limits by the capacity to dissipate heat might lie above the limits by mammary glands. Although other rodent species also showed inconsistent responses to the limitation on SusEI, including Brandt's voles [22,27], house mice *Mus musculus* [41,42], rats *Rattus norvegicus* [43] and cotton rats *Sigmodon hispidus* [4], we did not know if the different levels of limitation on SusEI also occur in these species. From these data, we suggest that the limitations on SusEI during peak lactation may not be the same in all species, even between different strains of mice, and we are currently testing how this would work.

In summary, shaved Swiss mice had higher energy intake and RMR during late lactation than non-shaved females. Dorsal fur removal did not have significant effects on litter size, litter mass, MEO, serum prolactin levels and suckling behavior. These data were inconsistent with the prediction by heat dissipation limitation hypothesis and provided support for the peripheral limitation hypothesis, whereby SusEI was more likely peripherally caused by the capacity of the mammary gland to produce milk.

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